AMENDMENTS TO THE SPECIFICATION

Page 1, between lines 3 and 4, please insert the following paragraph:

This application is a Continuation of U.S. Application Serial No. 09/971,611, filed on October 9, 2001.

Page 3, paragraph beginning at line 1 to line 9, delete in its entirety and insert therefor:

In one aspect of the present invention, there is thus provided a mutant α-amylase which is derived from an α-amylase having an amino acid sequence represented by ~~SEQ ID No. 1~~ SEQ ID No. 3 or showing at least 60% homology thereto by substitution or deletion of at least one amino acid residue corresponding to any one of Pro_{18}, Gln_{86}, Glu_{130}, Asn_{154}, Arg_{171}, Ala_{186}, Glu_{212}, Val_{222}, Tyr_{243}, Pro_{260}, Lys_{269}, Glu_{276}, Asn_{277}, Arg_{310}, Glu_{360}, Gln_{391}, Trp_{439}, Lys_{444}, Asn_{471} and Gly_{476} of the amino acid sequence.

Page 3, paragraph beginning at line 10 to line 16, delete in its entirety and insert therefor:

In another aspect of the present invention, there is also provided a mutant α-amylase derived from an α-amylase having an amino acid sequence represented by ~~SEQ ID No. 2~~ SEQ ID No. 4 or showing at least 60% homology thereto by substitution or deletion of at least one amino acid residue corresponding to any one of Asp_{128}, Gly_{140}, Ser_{144}, Arg_{168}, Asn_{181}, Glu_{207}, Phe_{272}, Ser_{375}, Trp_{434} and Glu_{466} of the amino acid sequence.

Page 4, paragraph beginning at line 16 to page 5, line 20, delete in its entirety and insert therefor:

The mutant α-amylase of the present invention is constructed so that out of amino acids constituting the α-amylase, the amino acid residues taking part in the productivity are substituted with another amino acid residues or deleted. Examples of the α-amylase usable here include liquefying α-amylases derived from *Bacillus. amyloliquefaciens* or *Bacillus.*

licheniformis and liquefying alkaline α-amylases derived from alkaliphilic microorganisms belonging to the *Bacillus* sp., of which α-amylases having an amino acid sequence represented by ~~SEQ ID No. 1 or SEQ ID No. 2~~ SEQ ID No. 2 or SEQ ID No. 4 and α-amylases having at least 60% homology to the above-described amino acid sequence are preferred.

Page 5, paragraph beginning at line 5 to line 12, delete in its entirety and insert therefor:

Examples of the α-amylase having the amino acid sequence represented by ~~SEQ ID No. 1~~ SEQ ID NO. 2, or α-amylase having at least 60% homology thereto include liquefying alkaline α-amylases derived from the strain *Bacillus* sp. KSM-AP1378 (FERM BP-3048) (Japanese Patent Application Laid-Open No. Hei 8-336392) and improved enzymes of the above-described one in heat resistance and oxidant resistance which are constructed by protein engineering technique (WO98/44126).

Page 5, paragraph beginning at line 13 to line 19, delete in its entirety and insert therefor:

Examples of the α-amylase having the amino acid sequence represented by ~~SEQ ID No. 2~~ SEQ ID No. 4 or having at least 60% homology thereto include liquefying alkaline α-amylases derived from the strain *Bacillus* sp. KSM-K38 (FERM BP-6946) and improved enzymes of the above-described one in heat resistance which are constructed by protein engineering technique (Japanese Patent Application No. Hei 11-163569).

Page 5, paragraph beginning at line 22 to page 6, line 9, delete in its entirety and insert therefor:

The mutant α-amylase of the present invention can be obtained first by cloning, from a microorganism producing an α-amylase, a gene encoding the α-amylase. For this purpose, ordinarily employed gene recombinant technique, for example, the method as described in

Japanese Patent Application Laid-Open No. Hei 8-336392 may be employed. Examples of the gene usable here include that represented by ~~SEQ ID No. 3 or SEQ ID No. 4~~ SEQ ID No. 1 or SEQ ID No. 3 which encodes the amino acid sequence represented by ~~SEQ ID No. 1 or SEQ ID No. 2~~ SEQ ID No. 2 or SEQ ID No. 4. Mutant genes derived from the above-described ones and having improved heat resistance and oxidant resistance are also usable.

Page 6, paragraph beginning a line 15 to page 8, line 11, delete in its entirety and insert therefor:

Mutation for obtaining highly productive α-amylases of the invention can be attained, for example, by substitution or deletion, in an a-amylase having an amino acid sequence represented by ~~SEQ ID No. 1~~ SEQ ID No. 2 or having at least 60% homology thereto, of at least one amino acid residue corresponding to any one of Pro_{18}, Gln_{86}, Glu_{130}, Asn_{154}. Arg_{171}, Ala_{186}, Glu_{212}, Val_{222}, Tyr_{243}, Pro_{260}, Lys_{269}, Glu_{276}, Asn_{277}, Arg_{310}, Glu_{360}, Gln_{391}, Trp_{439}, Lys_{444}, Asn_{471} and Gly_{476} of the amino acid sequence; or by substitution or deletion, in another α-amylase having an amino acid sequence represented by ~~SEQ ID No. 2~~ SEQ ID No. 4 or having at least 60% homology thereto, of at least one amino acid residue corresponding to any one of Asp_{128}, Gly_{140}, Ser_{144}, Arg_{168}, Asn_{181}, Glu_{207}, Phe_{272}, Ser_{375}, Trp_{434} and Glu_{466} of the amino acid sequence. Preferred mutations include, in the amino acid sequence of ~~SEQ ID No. 1~~ SEQ ID No. 2 substitution of the amino acid residue corresponding to Pro_{18} with Ser or Thr, the amino acid residue corresponding to Gln_{86} with Glu, the amino acid residue corresponding to Glu_{130} with Val or Gln, the amino acid residue corresponding to Asn_{154} with Asp, the amino acid residue corresponding to Arg_{171} with Cys or Gln, the amino acid residue corresponding to Ala_{186} with Val or Asn, the amino acid residue corresponding to Glu_{212} with Asp, the amino acid residue corresponding to Val_{222} with Glu, the amino acid residue corresponding to Tyr_{243} with Cys or Ser, the amino acid residue corresponding to Pro_{260} with Glu, the amino acid residue corresponding to Lys_{269} with Gln, the amino acid residue

corresponding to Glu_{276} with His, the amino acid residue corresponding to Asn_{277} with Ser or Phe, the amino acid residue corresponding to Arg_{310} with Ala, the amino acid residue corresponding to Glu_{360} with Gln, the amino acid residue corresponding to Gln_{391} with Glu, the amino acid residue corresponding to Trp_{439} with Arg, the amino acid residue corresponding to Lys_{444} with Arg, the amino acid residue corresponding to Asn_{471} with Asp or Glu, or the amino acid residue corresponding to Gly_{476} with Asp;

or substitution, in the amino acid sequence of ~~SEQ ID No. 2~~ SEQ ID No. 4, of the amino acid residue corresponding to Asp_{128} with Val or Gln, the amino acid residue corresponding to Gly_{140} with Ser, the amino acid residue corresponding to Ser_{144} with Pro, the amino acid residue corresponding to Arg_{168} with Gln, the amino acid residue corresponding to

Gln_{181} with Val, the amino acid residue corresponding to Glu_{270} with Asp, the amino acid residue corresponding to Phe_{272} with Ser, the amino acid residue corresponding to Ser_{375} with Pro, the amino acid residue corresponding to Trp_{434} with Arg or the amino acid residue corresponding to Glu_{466} with Asp.

Page 8, paragraph beginning at line 12 to page 9, line 9, delete in its entirety and insert therefor:

Among the mutations of the amino acid sequence of ~~SEQ ID No. 1~~ SEQ ID No. 2, those by substitution of the amino acid residue corresponding to Gln_{86} with Glu, the amino acid residue corresponding to Glu_{130} with Val or Gln, the amino acid residue corresponding to Ala_{186} with Asn, the amino acid residue corresponding to Tyr_{243} with Ser, the amino acid residue corresponding to Pro_{260} with Glu, the amino acid residue corresponding to Lys_{269} with Gln, the amino acid residue corresponding to Asn_{277} with Phe and the amino acid residue corresponding to Asn_{471} with Asp or Glu can bring about improvement in solubility of the α-amylase in a culture medium or desalted and concentrated solution thereof. More specifically,

the above-described mutations make it possible to improve the residual activity of the α-amylase in the supernatant after storage at 4°C for one week in a desalted and concentrated solution by at least 5%, especially 10% compared with the activity before mutation. Accordingly, in the case of the mutant α-amylases of the present invention obtained by such amino acid mutations, a fermented concentrate solution of a high concentration is available at a high yield and enzyme formulation treatment such as ultrafiltration after fermentation production can be conducted efficiently.

Page 9, paragraph beginning at line 10 to page 10, line 4, delete in its entirety and insert therefor:

A combination of two or more substitutions or deletions of various amino acid residues is also effective for such amino acid mutations. It is also possible to use the above-exemplified mutation in combination with a mutation for improving enzymatic properties, for example, in an a-amylase having an amino acid sequence represented ~~SEQ ID No. 1~~ SEQ ID No. 2 or having at least 60% homology thereto, a mutation for improving heat resistance by deleting amino acid residues corresponding to Arg_{181} and Gly_{182}, a mutation for improving oxidant resistance by substituting the amino acid residue corresponding to Met_{222} with Thr or a mutation for improving solubility by substituting the amino acid residue corresponding Lys_{484} with Gln; or in an α-amylase having an amino acid sequence represented by ~~SEQ ID No. 2~~ SEQ ID No. 4 or having at least 60% homology thereto, a mutation for further reinforcing oxidant resistance by substituting the amino acid residue corresponding to Met_{107} with Leu or a mutation for heightening detergency of a laundry detergent by substituting the amino acid residue corresponding Glu_{188} with Ile.

Page 18, paragraph beginning at line 23 to page 19, line 20, delete in its entirety and insert therefor:

In accordance with the method as described in WO98/44126, genes encoding a mutant α-amylase (which will hereinafter be described as "ΔRG") having improved heat resistance and a mutant α-amylase ("ΔRG-M202T") having improved oxidant resistance as well as improved heat resistance were constructed, respectively, by deleting Arg_{181} and Gly_{182} of the α-amylase ("LAMY") which was derived from the strain *Bacillus* sp. KSM-AP1378 (FERM BP-3048) and had the amino acid sequence represented by ~~SEQ ID No. 1~~ SEQ ID No. 2; and by, in addition to this mutation by deletion, substituting Thr for Met_{202} of the amino acid sequence represented by ~~SEQ ID No. 1~~ SEQ ID No. 2. With the genes as a template, gene fragments (about 1.5 kb) encoding these mutant α-amylases were amplified by the PCR reaction using primers LAUS (SEQ ID No. 5) and LADH (SEQ ID No. 6). After cutting of them with a restriction enzyme SalI, each of the fragments was inserted into the SalI-SmaI site of an expression vector pHSP64 (Japanese Patent Application Laid-Open No. Hei 6-217781), whereby a recombinant plasmid having a structural gene of each of the mutant α-amylases bonded thereto was constructed downstream of a strong promoter derived from an alkaline cellulase gene of the strain *Bacillus* sp. KSM-64 (FERM P-10482).

Page 19, paragraph beginning at line 21 to page 20, line 15, delete in its entirety and insert therefor:

In the meantime, with a chromosomal DNA, which had been extracted from the cells of the strain *Bacillus* sp. KSM-K38 (FERM BP-6946) by the method of Saito and Miura (Biochim. Biophys. Acta, **72**, 619 (1961)), as a template, PCR reaction was effected using primers K38US (SEQ ID No. 7) and K38DH (SEQ ID No. 8) shown in Table 2, whereby a structural gene fragment (about 1.5kb) encoding an α-amylase (which will hereinafter be described as "K38AMY") having an amino acid sequence of ~~SEQ ID No. 2~~ SEQ ID No. 4 was amplified. After cutting of it with a restriction enzyme SalI, the resulting fragment was inserted into the SalI-SmaI site of an expression vector pHSP64 to construct, downstream of

a strong promoter derived from an alkaline cellulase gene of the strain *Bacillus* sp. KSM-64 (FERM P-10482) contained in pHSP64, a recombinant plasmid having a structural gene of K38AMY bonded thereto (FIG. 1). With this recombinant plasmid as a template, PCR reaction was effected using the primers CLUBG (SEQ ID. No. 9) and K38DH (SEQ. ID. 8) to amplify a gene fragment (about 2.1 kb) having the strong promoter and K38AMY bonded thereto.

Page 20, paragraph beginning at line 16 to page 21, line 17, delete in its entirety and insert therefor:

By the recombinant PCR method as described below, a gene encoding chimeric α-amylase between K38AMY and LAMY was constructed. Described specifically, with a chromosomal DNA of the strain KSM-K38 (FERM BP6946) as a template, PCR reaction was conducted using primers K38DH (SEQ ID No. 8) and LA-K38 (SEQ ID No. 10) shown in Table 2, whereby a fragment encoding the sequence from Gln_{20} downstream to the C-terminal of the amino acid sequence of K38AMY represented by ~~SEQ ID No. 2~~ SEQ ID No. 4 was amplified. With the above-described recombinant plasmid containing the LAMY gene and strong promoter as a template, PCR reaction was conducted using primers CLUBG (SEQ ID No. 9) and LA-K38R (SEQ ID No. 11) shown in Table 2, whereby a gene fragment encoding from the upstream strong promoter to Gly_{21} of the amino acid sequence of LAMY of ~~SEQ ID No. 1~~ SEQ ID No. 2 was amplified. By the second PCR reaction using the resulting two DNA fragments and primers CLUBG (SEQ ID No. 9) and K38DH (SEQ ID No. 8) shown in Table 2, the resulting two fragments having, at the end thereof, complementary sequences derived from primers LA-K38 (SEQ ID No. 10) and LA-K38R (SEQ ID No. 11) respectively were combined, whereby a gene fragment (about 2.1kb) encoding a chimeric α-amylase (which will hereinafter be described as "LA-K38AMY") which has, successively

bonded thereto, a region encoding from His_1 to Gly_{21} of the LAMY downstream of the strong promoter and a region encoding from Gln_{20} to the C-terminal of the K38AMY was amplified.

Page 21, paragraph beginning at line 18 to page 23, line 6, delete in its entirety and insert therefor:

By using a "Site-Directed Mutagenesis System MutanSuper Express Km" kit (product of Takara Shuzo Co., Ltd.), the below-described mutations were introduced to the K38AMY and LA-K38AMY. First, the K38AMY and LA-K38AMY gene fragments (about 2.1kb) were inserted into the site SmaI of a plasmid vector pKF19k attached to the kit to construct a mutagenic recombinant plasmid (FIG. 2). A site-directed mutagenic oligonucleotide primer N190F (SEQ ID No. 50) shown in Table 2 was 5'-phosphorylated with T4 DNA kinase. Using this and the above-described mutagenic recombinant plasmid, mutagenesis was effected in accordance with the method of the kit and by using the reaction product, the strain *Escherichia coli* MV1184 ("Competent cell MV1184", product of Takara Shuzo Co., Ltd.) was transformed. From the transformant thus obtained, a recombinant plasmid was extracted, followed by analysis of a basic sequence, whereby mutation by substitution of Phe for Asn_{190} was confirmed. By repeated introduction of mutagenic reactions into the mutated gene by successively using primers A209V (SEQ ID No. 51) and QEYK (SEQ ID No. 49) in a similar manner as above, thereby substituting Asn_{190} and Gln_{209}, each of the amino acid sequence of the K38AMY represented by ~~SEQ ID No. 2~~ SEQ ID No. 4 with Phe and Val, respectively, and the sequence from Asp_1 to Gly_{19} of the amino acid sequence of the K38AMY represented by ~~SEQ ID No. 2~~ SEQ ID No. 4 with the sequence from His_1 to Gly_{21} of the amino acid sequence of the LAMY represented by ~~SEQ ID NO. 1~~ SEQ ID No. 2; by substituting Gln_{167}, Tyr_{169}, Asn_{190} and Gln_{209}, each of the amino acid sequence of the K38AMY, with Glu, Lys, Phe and Val, respectively and the sequence from Asp_1 to Gly_{19} of the amino acid sequence of the K38AMY with the sequence from His_1 to

Gly_{21} of the amino acid sequence of the LAMY; and substituting Gln_{167} and Tyr_{169}, Asn_{190} and Gln_{209}, each of the amino acid sequence of the K38AMY, with Glu, Lys, Phe and Val, respectively, genes encoding a mutant α-amylase (which will hereinafter be described as "LA-K38AMY/NFQV") having improved heat resistance, a mutant α-amylase ("LA-K38AMY/QEYK/NFQV") having drastically improved heat resistance, and a mutant α-amylase ("QEYK/NFQV") having improved heat resistance were constructed, respectively.

Please delete the original Sequence Listing appearing on pages 38-65.

Page 69 (Abstract), after the last line, beginning on a new page, please insert the attached substitute Sequence Listing.